Atty. Docket No.: 4231/2002 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Liew, C.C.	Examiner:	Juliet C. Switzer
Serial No.:	10/085,783		
Filed:	Feb. 28, 2002	Group Art Unit:	1634
Titled:	COMPOSITIONS AND METHODS RELATING TO OSTEOARTHRITIS	Conf. No.:	8718

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL DECLARATION OF HONGWEI ZHANG UNDER U.S.C. 1.132 TO THE DECLARATION DATED JULY 17, 2006

Sir:

I, **Hongwei Zhang**, Ph.D., hereby declare that:

1. I received a Ph.D. degree from the Institute of Medical Science at the University of Toronto in 2002, and a Master of Science degree from the Department of Immunology at the University of Toronto in 1995. In addition I received my Medical Degree from the University of Medical Sciences in Changchun China in 1989 and practiced as a staff physician for 4 years in Beijing prior to commencing my post graduate studies. I currently hold the positions of Senior Scientist and Scientific Program Leader of Functional Genomics as well as Manager of Research and Development at GeneNews Corporation.

I am one of the inventors of the above-noted U.S. patent application.

I am particularly experienced in the field of osteoarthritis having worked as a Research Associate at the Arthritis Center of Excellence of Toronto Western Hospital, and subsequently receiving a Fellowship from the institute to pursue my PhD studies focusing on the area of osteoarthritis. Subsequently I have been one of the key scientists involved in the ongoing collaboration of GeneNews (formerly ChondroGene) with Pfizer in the area of osteoarthritis and

biomarker discovery. I am a trained molecular biologist experienced in developing methods to identify biomarkers which are indicative of a disease or condition, and in developing methods of using these biomarkers and products thereof as applied in the area of osteoarthritis, amongst other conditions.

List of Publications:

K.W. Marshall, M.D., PhD., F.R.C.S., **H. Zhang, M.D., PhD.**, T.D. Yager Ph.D., N. Nossova M.D., Ph.D., A. Dempsey PhD., R. Zheng M.D., M. Han M.D. Ph.D., H.Tang M.Sc., S. Chao M.A.Sc, and C.C. Liew PhD. "Blood-based biomarkers for detecting mild osteoarthritis in the human knee" *OsteoArthritis and Cartilage (2005)* 861-871.

Zhang H, Marshall KW, Tang H, Hwang DM, Lee M, Liew CC. Profiling genes expressed in human fetal cartilage using 13,155 expressed sequence tags. Osteoarthritis Cartilage 2003;11:309-19.

Hongwei Zhang, C.C.Liew, K.Wayne Marshall. Microarray Analysis Reveals the Involvement of Beta-2 Microglobulin (B2M) in Human Osteoarthritis. Osteoarthritis and Cartilage 2002;10:950-60.

Doherty PJ, **Zhang H**, Manolopoulos V, Trogadis J, Tremblay L, Marshall KW. Adhesion of transplanted chondrocytes onto cartilage in vitro and in vivo. J Rheumatol 2000;27:1725-312.

Zhao YX, Lajoie G, **Zhang H,** Chiu B, Payne U, Inman RD. Tumor necrosis factor receptor p55-deficient mice respond to acute Yersinia enterocolitica infection with less apoptosis and more effective host resistance. Infect Immun 2000;68:1243-513.

Vaselios Manolopoulos, K. Wayne Marshall, **Hongwei Zhang**, Judy Trogadis, Louise Trembly and Paul J. Doherty. Factors affecting the efficacy of bovine chondrocyte transplantation in vitro. Osteoarthritis and Cartilage 1999;7:453-460.

Yi-Xue Zhao, **Hongwei Zhang**, Basil Chiu, Usulra Payne, Robert D. Inman. Tumor necrosis factor receptor P55 controls the severity of arthritis in experimental *Yersinia Enterocolitica* infection. Arthritis & Rheumatism 1999;42:1662-1672.

Paul J. Doherty, **Hongwei Zhang**, Louise Trembley, Vaselios Manolopoulos and K. Wayne Marshall. Resurfacing of articular cartilage explants with genetically-modified human chondrocytes *in vitro*. Osteoarthritis and Cartilage 1998;6:153-160.

Hongwei Zhang, Donna Phang, Ronald M. Laxer, Earl D. Silverman, Sueihua Pan, and Paul J. Doherty. Evolution of the T cell receptor beta repertoire from synovial fluid T cells of patient with juvenile onset rheumatoid arthritis. J. Rheumatol. 1997;24:1396-402.

Petro Lastres, Anihoa Letamendia, **Hongwei Zhang**, Carlos Rius, Nuria Almendro, UIIa RAab, Louis A. Lopez, Carmen Langa, Angels Fabra, Michelle Letarte and Carmelo Bernabeu. Endoglin modulates cellular responses to TGF-beta 1. J. Cell Biol. 1996;133:1109-1121.

Hongwei Zhang, Andrew R.E. Shaw, Allan Mak, and Michelle Letarte. Endoglin is a component of the Transforming Growth Factor (TGF)-beta receptor complex of human pre-B leukemic cells. J. Immunol. 1996,156:565-573.

2. I have read the final Office Action mailed October 2, 2006 and the Advisory Action mailed January 29, 2007 in the above-referenced patent application.

In providing grounds for rejection of claims 58, 60, 66, 68 and 73-76 under 35 U.S.C. § 112(1), the Examiner asserts (at page 12 of the Office Action) that the specification does not provide an example of "*an expression pattern containing the three elected genes that has been determined using routine statistical methods to be present more often in patients with disease than without disease*". The Examiner asserts that though the declaration filed 7/17/06 ("2nd declaration") begins to provide the relevant data, it remains insufficient to support such an assertion.

3. As a scientist skilled in the area of osteoarthritis and molecular biomarker identification, I submit that the three elected genes (TNFAIP6, LAMC1 and CALM1) have been experimentally shown to exhibit a statistically significant differential expression pattern in cartilage of patients having osteoarthritis (OA) relative to subjects not having OA.

Alternate statistical analysis of ChondroChip Hybridization Data

Attached as Exhibit "A" to this Declaration are summarized results of a data analysis clearly showing that TNFAIP6, LAMC1 and CALM1 exhibit a statistically significant differential expression pattern in cartilage of patients having OA relative to subjects not having OA.

The results shown in Exhibit "A" were obtained from an alternate statistical analysis of the same ChondroChip^{TM} microarray hybridization data described in the declaration dated july 17, 2006, the latter showing that each of the three elected genes is differentially expressed in OA cartilage relative to non-OA cartilage (i.e. TNFAIP6 and CALM1, up-regulated; and LAMC1, down-regulated in OA versus normal cartilage). We have re-analyzed data representing expression profiles of the three elected genes in cartilage samples from 6

individuals not having OA (healthy) and 19 individuals having OA (8 moderate and 11 severe OA) so as to characterize the global expression pattern of the three elected genes in samples derived from single individuals having or not having OA. Using MEDCALC software, expression level data for each gene was analyzed via the ROC curve approach to determine the best expression level threshold to differentiate between expression levels in OA cartilage and in normal cartilage. The expression levels for each gene for each sample were assigned a supra- or sub-threshold value, and values corresponding to OA or to normal/non-OA relative to threshold were assigned a "threshold score" of "1" or "0", respectively. For each sample, the threshold scores for the three genes were added to generate a "classification value" serving to classify the sample as being derived from an individual having OA or being normal. Namely, samples having a threshold score of "1" for each of the three genes were assigned a classification value of "1" classifying the sample as being derived from OA cartilage, and samples comprising one or more "0" threshold scores were conservatively assigned a classification value of "0" classifying the sample as being derived from non-OA cartilage. It can be seen that 6 of the 19 samples from OA patients, but none of the 6 samples from the non-OA subjects, exhibit the instantly claimed differential gene expression pattern for the three elected genes, i.e. it can be seen that TNFAIP6 and CALM1 are up-regulated, and that LAMC1 is down-regulated in cartilage from individuals having OA relative to cartilage from individuals not having OA.

In view of the above, I submit that the specification enables one of one skill in that art to practice the claimed methods.

4. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Hongwei Zhang, Ph.D. _____

Date Oct. 29, 2007

EXHIBIT "A"

Sample ID	Fluorescence intensity (relative mRNA level)			Threshold Score			Classification Value (0: non-OA; 1: OA)
	TNFAIP6	LAMC1	CALM1	TNFAIP6 (0: ≤ 1.261, 1: >1.261)	LAMC1 (0: >0.655, 1: ≤0.655)	CALM1 (0: ≤1.301, 1: >1.301)	
Normal, N111	0.589	0.987	0.518	0	0	0	0
Normal, N103	0.751	0.703	0.465	0	0	0	0
Normal, N106	0.814	0.661	0.686	0	0	0	0
Normal, N108	0.9	0.956	0.536	0	0	0	0
Normal, N110	0.974	0.883	0.654	0	0	0	0
Normal, N101	1.261	0.611	1.301	0	1	0	0
Moderate OA, Mod779A normalized	0.882	0.787	2.145	0	0	1	0
Severe OA, S426 normalized	0.882	0.384	1.467	0	1	1	0
Moderate OA, Mod634 normalized	0.954	0.649	2.027	0	1	1	0
Moderate OA, Mod850B normalized	1.068	0.571	1.565	0	1	1	0
Moderate OA, Mod890 normalized	1.079	0.695	2.948	0	0	1	0
Moderate OA, Mod882B normalized	1.109	0.632	2.114	0	1	1	0
Severe OA, S282 normalized	1.35	0.503	2.681	1	1	1	1
Severe OA, S440 normalized	1.533	0.627	1.99	1	1	1	1
Severe OA, S393 normalized	1.654	0.447	1.379	1	1	1	1
Severe OA, S201 normalized	1.689	0.612	1.227	1	1	0	0
Moderate OA, Mod602 normalized	1.805	0.677	2.395	1	0	1	0
Moderate OA, Mod699 normalized	1.85	1.185	2.393	1	0	1	0
Severe OA, S622 normalized	1.925	0.655	1.384	1	0	1	0
Severe OA, S199 normalized	2.018	0.706	1.752	1	0	1	0
Moderate OA, Mod350 normalized	2.487	0.585	2.228	1	1	1	1
Severe OA, Sev650 normalized	2.78	0.638	1.717	1	1	1	1
Severe OA, S266 normalized	3.762	0.653	2.17	1	1	1	1
Severe OA, S855 normalized	7.016	0.551	0.953	1	1	0	0
Severe OA, S821B normalized	7.985	0.714	1.616	1	0	1	0

Summary of data:

	TNFAIP6	LAMC1	CALM1	3 genes combined
True negatives	6/6	5/6	6/6	6/6
True positives	13/19	12/19	17/19	6/19
Accuracy	19/25	17/25	23/25	12/25
Specificity	100%	83%	100%	100%
Sensitivity	68%	63%	89%	32%
Fold-change (OA/non-OA)	2.6	0.80	2.7	–
p-value	<0.0001	0.0268	<0.0001	–